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Form Approved Through
OMB No. 0930-0045

DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE		LEAVE BLANK FOR PHS USE ONLY.	
GRANT APPLICATION		Type	Activity
Follow instructions carefully. Type in the unshaded areas only. Type density must be 10 c.p.i.		Review Group	Number
		Council/Board (Month, Year)	Formerly
			Date Received
1. TITLE OF PROJECT (Do not exceed 55 typewriter spaces.) Cardiotoxicity of Streptococcal Pyrogenic Exotoxins			
2a. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT		<input checked="" type="checkbox"/> NO <input type="checkbox"/> YES (If "YES," state number and)	
Number: Title:			
2b. TYPE OF GRANT PROGRAM		3. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR	
3a. NAME (Last, first, middle) Schlievert, Patrick M.		3b. DEGREE(S) Ph.D.	3c. SOCIAL SECURITY NO. 485-56-9034
3d. POSITION TITLE Professor		3e. MAILING ADDRESS (Street, city, state, zip code) Department of Microbiology University of Minnesota Box 196 UMHC 420 Delaware Street, SE Minneapolis, MN 55455-0312	
3f. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT Microbiology			
3g. MAJOR SUBDIVISION Medical School			
3h. TELEPHONE AND FAX (Area code, number and extension) TEL: 612-624-1484 FAX: 612-626-0623		BITNET/INTERNET ADDRESS micro@levent.med.umn.edu	
4. HUMAN SUBJECTS		5. VERTEBRATE ANIMALS	
4a. <input checked="" type="checkbox"/> NO <input type="checkbox"/> YES		5a. <input type="checkbox"/> NO <input checked="" type="checkbox"/> YES	
4b. Assurance of compliance no.		5b. Assurance of compliance no.	
6. DATES OF ENTIRE PROPOSED PROJECT PERIOD From (MMDDYY) Through (MMDDYY)		7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD 7a. Direct Costs (\$) 89,687 7b. Total Costs (\$) 125,562	
		8. COSTS REQUESTED FOR ENTIRE PROPOSED PROJECT PERIOD 8a. Direct Costs (\$) 279,967 8b. Total Costs 391,954	
9. PERFORMANCE SITES (Organizations and addresses) 960 Mayo Building University of Minnesota 420 Delaware Street SE Minneapolis, MN 55455		10. INVENTIONS AND PATENTS (Completing continuation application only) <input checked="" type="checkbox"/> NO <input type="checkbox"/> YES "YES," Previously reported <input type="checkbox"/> Not previously reported	
11. NAME OF APPLICANT ORGANIZATION University of Minnesota ADDRESS Research Technology Transfer Admin 1100 Washington Avenue S, Ste. 201 Minneapolis, MN 55415-1226		12. TYPE OF ORGANIZATION <input checked="" type="checkbox"/> Public: Specify <input type="checkbox"/> Federal <input checked="" type="checkbox"/> State <input type="checkbox"/> Local <input type="checkbox"/> Private Nonprofit <input type="checkbox"/> Forprofit (General) <input type="checkbox"/> Forprofit (Small Business)	
13. ENTITY IDENTIFICATION NUMBER 1-416007513-A8		14. BIOMEDICAL RESEARCH SUPPORT GRANT CREDIT Code: 01 Identification: School of Medicine	
15. NAME OF ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE A.R. Potami TELEPHONE 612-624-1648 FAX 612-624-4843 TITLE Associate Vice President ADDRESS ORTTA 1100 Washington Avenue S, Ste 201 Minneapolis, MN 55415-1226 BITNET/INTERNET ADDRESS arpotami@ortta.umn.edu		16. NAME OF OFFICIAL SIGNING FOR APPLICANT ORGANIZATION A.R. Potami or Mary Lou Weiss TELEPHONE 612-624-5856 FAX 612-624-4843 TITLE Assistant Director ADDRESS ORTTA 1100 Washington Avenue S., Ste. 201 Minneapolis, MN 55415-1226 BITNET/INTERNET ADDRESS mweiss@ortta.umn.edu	
17. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application. Willful provision of false information is a criminal offense (U.S. Code, Title 18, Section 1001). I am aware that any false, reckless, or fraudulent statement may, in addition to other remedies available to the Government, subject me to civil penalties under the Program Fraud Civil Remedies Act of 1986 (45 CFR 79).		SIGNATURE OF PERSON NAMED IN 3a. (In ink - "Per" signature not acceptable.) Patrick Schlievert	
18. CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true and complete to the best of my knowledge, and accept the obligation to comply with Public Health Service terms and conditions if a grant is awarded as a result of this application. A willful false certification is a criminal offense (U.S. Code, Title 18, Section 1001). I am aware that any false, reckless, or fraudulent statement may, in addition to other remedies available to the Government, subject me to civil penalties under the Program Fraud Civil Remedies Act of 1986 (45 CFR 79).		SIGNATURE OF PERSON NAMED IN 16. (In ink - "Per" signature not acceptable.)	

DESCRIPTION: State the application's broad, long-term objectives and specific aims, making reference to the health relatedness of the project. Describe concisely the research design and methods for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. This abstract is meant to serve as a succinct and accurate description of the proposed work when separated from the application. DO NOT EXCEED THE SPACE PROVIDED.

The long term goals of this project are two-fold: (a) to evaluate the role of streptococcal pyrogenic exotoxins (SPEs, scarlet fever toxins) in causing both acute TSS-like illnesses and delayed sequelae such as acute rheumatic fever, guttate psoriasis, and vascular diseases, and (b) to analyze the structure-function relationships among the SPEs and between the SPEs and staphylococcal enterotoxins and TSST-1. It is hoped that the latter studies will ultimately clarify the molecular mechanism of action of the toxins.

Experiments will be done to prepare sufficient SPEs A and C for crystallization and three-dimensional structure determination. Toxin purification will be accomplished by ethanol precipitation from large cultures, toxin resolubilization in pH 4.0 acetate-buffered saline, and preparative thin layer isoelectric focusing. Crystallization and three dimensional structure determination will be done in collaboration with Douglas H. Ohlendorf, Department of Biochemistry, University of Minnesota. Domains and amino acids on SPE types A and C required for biological activity (pyrogenicity, enhancement of lethal endotoxin shock, enhancement of cardiotoxicity, ability to induce TSS when administered subcutaneously in miniosmotic pumps, and superantigenicity) and lipopolysaccharide binding will be localized through use of PCR mutagenesis, both site specific and through deletion of entire domains. Nucleotide sequencing will be done to verify changed amino acids and structure determination will be done to assess alteration of 3-dimensional structure of mutants. Although not an objective, it is anticipated these latter studies will also localize domains recognized by monoclonal antibodies to the two SPEs.

PERSONNEL ENGAGED ON PROJECT, INCLUDING CONSULTANTS/COLLABORATORS. Use continuation pages as needed to provide the required information in the format shown below on all individuals participating in the scientific execution of the project.

Name	Patrick M. Schlievert	Degree(s)	Ph.D.	Social Security No.	485-56-9034
Position Title	Professor	Date of Birth (MM/DD/YY)	06/02/49	Role on Project	PI
Organization	University of Minnesota			Department	MICROBIOLOGY
Name	Douglas H. Ohlendorf	Degree(s)	Ph.D.	Social Security No.	328-44-8583
Position Title	Associate Professor	Date of Birth (MM/DD/YY)	09/19/50	Role on Project	Collaborator
Organization	University of Minnesota			Department	BIOCHEMISTRY
Name		Degree(s)		Social Security No.	
Position Title		Date of Birth (MM/DD/YY)		Role on Project	
Organization				Department	
Name		Degree(s)		Social Security No.	
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Organization				Department	
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Organization				Department	